New Approach Methods and Reducing the Use of Laboratory Animals for Chronic and Carcinogenicity Testing

Anna Lowit, Ph.D.
Science Advisor
US Environmental Protection Agency, Office of Pesticide Programs
Lowit.anna@epa.gov / +1-703-308-4135 / +1-703-258-4209
Consultation with the Science Advisory Board
June 24, 2020

USEPA Administrator Memo Prioritizing Efforts to Reduce Animal Testing, September 10, 2019

- EPA will reduce its requests for, and our funding of, mammal studies by 30 percent by 2025
- EPA will eliminate all mammal study requests and funding by 2035. Any mammal studies requested or funded by the EPA after 2035 will require Administrator approval on a case-by-case basis.
- https://www.epa.gov/environmental-topics/administrator-memo-prioritizing-efforts-reduce-animal-testing-september-10-2019
- New Approach Methods Work Plan released this week

OPP's Guidances & Policies for Granting Waivers

- 2013 Guiding Principles for Data Needs for Pesticides:
  - https://www.epa.gov/pesticide-registration/guiding-principles-data-requirements
  - "…ensure there is sufficient information to reliably support registration decisions that are protective of public health and the environment while avoiding the generation and evaluation of data that does not materially influence the scientific certainty of a regulatory decision…."
- Part 158 Toxicology Data Requirements: Guidance for Neurotoxicity Battery, Subchronic Inhalation, Subchronic Dermal and Immunotoxicity Studies
  - http://www.epa.gov/pesticides/regulating/part158-tox-data-requirement.pdf
  - >200,000 laboratory animals saved
  - >$300 million to industry saved
  - Craig E, Lowe K, Akerman G, et al. Reducing the need for animal testing while increasing efficiency in a pesticide regulatory setting: Lessons from the EPA Office of Pesticide Programs' Hazard and Science Policy Council. Regul Toxicol Pharmacol. 2019;108:104481. doi:10.1016/j.yrtph.2019.104481

OPP's Guidances & Policies for Granting Waivers

- OECD Guidance Document for Waiving or Bridging Acute Toxicity Tests
  - Co-authored by USEPA & Canada PMRA
  - Provides international guidance on waiving acute lethality studies for oral, dermal and inhalation
  - http://www.oecd.org/env/ehs/testing/mono%202016%2032.pdf
- Guidance for Waiving Acute Dermal Toxicity Tests for Pesticide Formulations & Supporting Retrospective Analysis
  - https://www.epa.gov/sites/production/files/2016-11/documents/acute-dermal-toxicity-pesticide-formulations_0.pdf
- Final Guidance for Waiving Sub-Acute Avian Dietary Tests for Pesticide Registration and Supporting Retrospective Analysis
  - https://www.epa.gov/sites/production/files/2020-02/documents/final-waiver-guidance-avian-sub-acute-dietary.pdf
  - Hilton, G.M., Odenkirchen, E., Panger, M., Waleko, G., Lowit, A., Clippinger, A.J. 2019, Regulatory Toxicology and Pharmacology, 105: 30-35, https://doi.org/10.1016/j.yrtph.2019.03.013

Other On-Going Efforts by OPP

- **Acute toxicity "6-pack" initiative-**
  - Currently have a policy in place to accept eye irritation assays for antimicrobial cleaning products: https://www.epa.gov/pesticide-registration/alternate-testing-framework-classification-eye-irritation-potential-epa
  - Evaluation of QSAR for acute oral LD_{50}; Collaborative Acute Toxicity Modeling Suite (CATMoS)
  - Activities to replace the in vivo eye irritation & dermal irritation studies
- **Inhalation**– use of *in vitro* model using chlorothanil as a case study– brought to SAP in 2018
- **Dermal Absorption "Triple Packs"** - Human in vitro, rat in vitro, and rat in vivo studies
- **Fish acute retrospective** - OPP is working with NICEATM to evaluate whether we reduce the number of studies we receive (typically, we receive 3 different species)

Skin Sensitization: Replacement of Laboratory Animal Testing

- Isothiazolinones: antimicrobial pesticides (biocides) that are positive skin sensitizers
  - Use as material preservative presents concern, as products containing these chemicals do not bear pesticide labels to communicate potential hazard to consumers
  - First use of in vitro data to derive point of departure for quantitative risk assessments of 6 isothiazolinones (draft risk assessments released May 14, 2020)
  - https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0159-0008
  - Collaborative work with the National Toxicology Program
  - Quantitative approach to assess potential skin sensitization by identifying induction and/or elicitation thresholds for each chemical to characterize risk from dermal exposure
  - Approach extends previously used principles for assessing skin sensitization potential by using in vitro and in chemico assays and neural network-based defined approaches (DAs)
  - Public comment period is open until August 13, 2020

Presentations

- Rethinking Carcinogenicity Assessment for Agrochemicals Project: Dr. Gina Hilton, People for the Ethical Treatment of Animals, International Science Consortium, Ltd.
- National Toxicology Program Efforts to Improve Carcinogenic Assessment of Environmental Substances: Dr. Warren Casey, National Institute of Environmental Health Sciences
- Health and Environmental Sciences Institute's Emerging Systems in Toxicology (HESI eSTAR): Transcriptomic Point of Departure Program: Dr. Jessica LaRocca, Corteva Agriscience and Dr. Scott Auerbach, National Institute of Environmental Health Sciences
- Gene Expression Evaluation of Pesticides with Established Liver Tumor Modes of Action, Dr. Chris Corton, EPA Office of Research and Development
- Kinetically-Derived Maximum Doses: Dr. Cecilia Tan, EPA Office of Pesticide Programs

ReCAAP: Rethinking Carcinogenicity Assessment for Agrochemicals Project

PETA INTERNATIONAL SCIENCE CONSORTIUM LTD.

Gina Hilton, PhD
PETA International Science Consortium Ltd.

EPA United States Environmental Protection Agency

Gregory Akerman, PhD
United States Environmental Protection Agency

EPA United States Environmental Protection Agency

Guiding Principles for Data Requirements (2013)

Alternative approaches can be accepted, and **studies can be waived** (§158.45), avoiding the generation and evaluation of data that does not materially influence the scientific certainty of a regulatory decision. **Only require data that adequately inform regulatory decision making**.

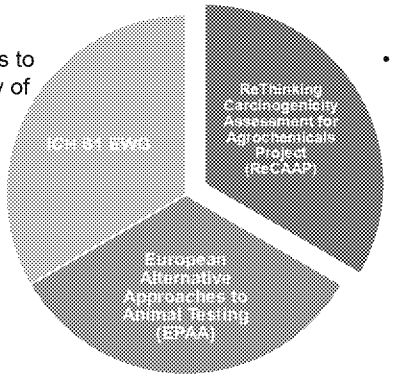
Part 158 Toxicology Data Requirements: Guidance for Neurotoxicity Battery, Subchronic Inhalation, Subchronic Dermal and Immunotoxicity Studies (2013)

Purpose: Provide guidance on the weight of the evidence-based (WOE) determination of data needs for neurotoxicity, subchronic inhalation, subchronic, dermal and immunotoxicity studies and provide guidance on how to consider the data needs determination in risk assessment.

https://www.epa.gov/pesticide-registration/determining-toxicology-data-requirements

International alternative weight of evidence-based approach for carcinogenicity assessment

- Alternative approaches to assess carcinogenicity of pharmaceuticals



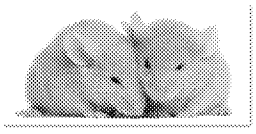
- Criteria to consider for waiving chronic/ carcinogenicity testing of agrochemicals
- Alternative approaches using mechanistic information to assess carcinogenicity of agrochemicals

ReThinking Carcinogenicity Assessment for Agrochemicals Project (ReCAAP)

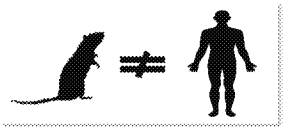
Goal: Develop a framework to determine when the rat and/or mouse cancer bioassays can be waived via a weight of evidence-based approach for food-use agrochemicals

Problem Statement

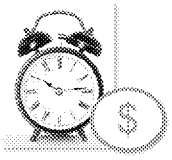
"There are no specific criteria to determine when not to require the Combined Chronic Toxicity/Carcinogenicity studies (OECD 453; 451), or how to determine appropriate POD for chronic risk assessments for pesticides based on available toxicological and exposure data in the absence of chronic toxicity studies…there is a movement to transition away from a routine 'check-box' approach towards a more scientifically sound weight of evidence (WOE) carcinogenicity assessment for non-genotoxic food-use pesticides."



Animal Welfare



Human Relevance



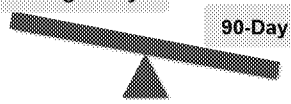
Cost

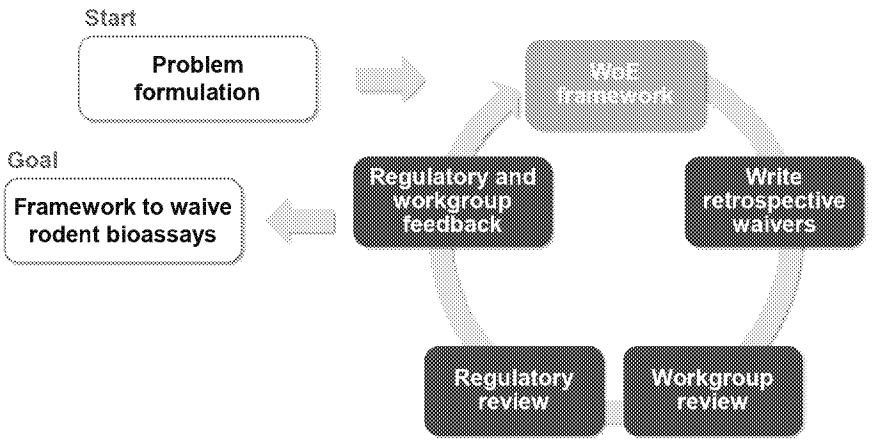
Check-box approach

Studies	Required
Acute Oral Toxicity	☒
Acute Dermal Toxicity	☒
Acute Inhalation Toxicity	☒
Acute Eye Irritation	☒
Acute Dermal Irritation	☒
Skin Sensitization	☒
90-Day Oral Toxicity in Rodents	☒
90-Day Oral Toxicity in Non-rodents	☒
21/28-Day Dermal Toxicity	☒
90-Day Dermal Toxicity	☐
90-Day Inhalation Toxicity	☒
Developmental Toxicity in Rodents	☒
Developmental Toxicity in Non-rodents	☒
Reproduction and Fertility Effects	☒
Chronic Toxicity in Rodents	☒
Chronic Toxicity in Non-rodents	☐
Carcinogenicity in Rats	☒
Carcinogenicity in Mice	☒

Weight of Evidence approach

- Read-across
- Metabolism
- Hormonal Effect
- Mechanistic
- Genotoxicity
- Exposure
- 28-Day
- 90-Day
- Carcinogenicity





Poster reference: https://bit.ly/3d44KOP

Draft Carcinogenicity Waiver Reporting Framework

I. Purpose of this Analysis
II. Study Waiver Requests
    1. Use Pattern and Exposure Scenarios
    2. Physical-Chemical Properties
    3. ADME and Toxicokinetics
    4. Toxicity
        4.1 Acute Toxicity
        4.2 Subchronic Toxicity
        4.3 Evidence of Hormone Perturbation
        4.4 Evidence of Immune Suppression
        4.5 Genetic Toxicity
        4.5 Special Studies and Endpoints
    5. Evidence of Chronic Toxicity from Related Chemicals
    6. Proposed Points of Departure, and Prospective Risk Assessments
    7. Conclusion
    8. References

Weight of Evidence	Case Study
Intended Use / Chemical Class / MOA	Herbicide safener; arylsulfonyl-benzamides; induce herbicide metabolizing enzymes
Physical-Chemical Properties	Molecular weight = 374.41 Vapor pressure = 6 x 10-9 Pa at 20°C Log Kow = -0.80
Use Pattern & Exposure Scenarios	Uses: corn, sorghum, turf, and ornamentals Exposure: human dietary
Acute Toxicity (EPA Category)	Oral (III); Dermal (III); Inhalation (III); Eye (IV); Dermal Irritation (IV); Skin Sensitization (Negative)
Subchronic Toxicity NOAEL (mg/kg/day)	28 day (dog): 92/314 (M/F) 90 day (mouse, rat, dog): 1110/398 (M/F), 58/70 (M/F), 221 (M/F) Primary results: lymphocytolysis in the thymus, kidney, and urinary tract. The urinary tract was the common target
Evidence of Hormone Perturbation	Offspring: pup body weight decrease Maternal: organ weight changes in spleen and urinary tract Reproductive: reduced rearing index Effects are unlikely to be due to a hormone-disruption mechanism

Weight of Evidence	Case Study
Evidence of Immune Suppression	No evidence of treatment-related immunotoxicity
Genetic Toxicity	Non-genotoxic
ADME	Rapidly absorbed and then rapidly excreted, primarily unchanged, and predominantly in the urine
Read-Across	1 sulfonamide antimicrobial, sulfanilamide chemical class, used for read-across based on structural similarity. Chemical showed similar toxicity via urinary calculi formation
Special Studies (Nuclear receptor activation)	Cytochrome P450 induction was investigated in M/F rats dosed up to 600 mg/kg/day for 14 days. No indication of induction of AhR, CAR, PXR, or PPARα nuclear receptors. PBPK model to determine the dietary chronic exposure level in humans that could lead to urinary concentrations. Negligible concern for tumor formation.

Weight of Evidence	Case Study
Summary of Chronic Toxicity/Carcinogenicity from Read-across Chemicals	• 1 read-across pharmaceutical chemical – Not a pesticide • The calculi-based mode of action is characterized by the toxic, proliferative, and tumorigenic effects, which only occur in the presence of calculi (under high dose conditions) • Read-across showed similar toxicity via urinary calculi formation. No additional concern for chronic or carcinogenic toxicity
Proposed chronic population adjusted dose (cPAD)	• 58 mg/kg/day = NOAEL from 90-day rat study • 1000X UF = total uncertainty factor (10X inter-species, 10X intra-species, 10X subchronic to chronic) • cPAD = 0.058 mg/kg/day • % cPAD = 0.4% (calculated with most sensitive exposure estimate) • 0.4% is below EPA level of concern

Proposed by waiver author: both the rat and the mouse carcinogenicity studies should be waived

Lessons learned: key workgroup feedback

Specific Feedback (this case study)

- Limited read-across information
- High dose argument
- PBPK models could be a very useful tool in weight-of-evidence. Software used for modeling needs to be open source
- Different agencies reviewing the documents have slightly differing opinions on some areas of the WoE which increases the complexity of the evaluations

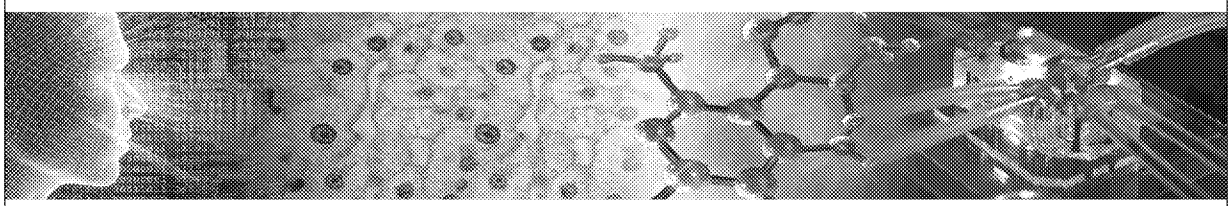
General Feedback

- Only use quantitative descriptors
- Include structural information for metabolites
- Literature search (if available)
- Read-across is a critical consideration in the weight-of-evidence assessment, and more detailed information should be provided

Conclusions

- ReCAAP provides a process to develop a weight of evidence framework to identify elements to consider when waiving the rat and/or mouse carcinogenicity tests for food-use pesticides while still protecting human health.
- Weight of evidence information includes, but is not limited to: estimated human exposure, subchronic toxicity, metabolism, mode of action/mechanistic data, and other critical components relevant to the protection of human health.
- The proposed framework has gone through several iterations of review and refinement – demonstrating a collaborative and iterative approach to develop case study waivers from currently registered pesticides.
- US EPA, Health Canada PMRA, and Australia APVMA are actively providing feedback on retrospective waivers to identify what information could be useful in a weight of evidence-based approach to support a waiver for rodent carcinogenicity testing.

NTP
National Toxicology Program



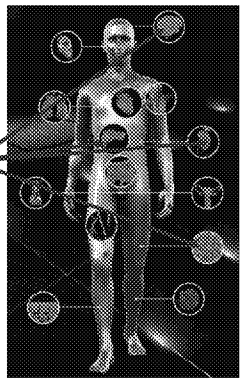
Advancing Carcinogenicity Assessment at the National Toxicology Program

Warren Casey, PhD, DABT
Chief (Acting), Biomolecular Screening Branch

Division of the National Toxicology Program
National Institute of Environmental Health Sciences

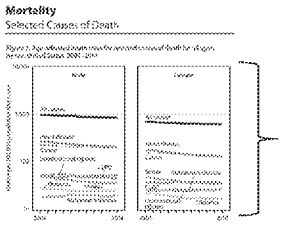
Fundamental shift in approach

X lbs./yr. commercial production

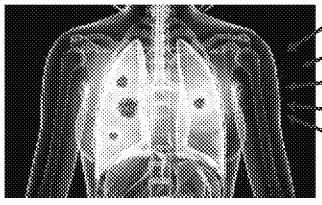


Agent Y

https://ncats.nih.gov/tissuechip/chip



Contemporary public health concerns



Agent A

Agent B

Agent C

Agent Y

Agent Z

Agent-focused → Disease-focused

NTP Health Effects Innovation Areas

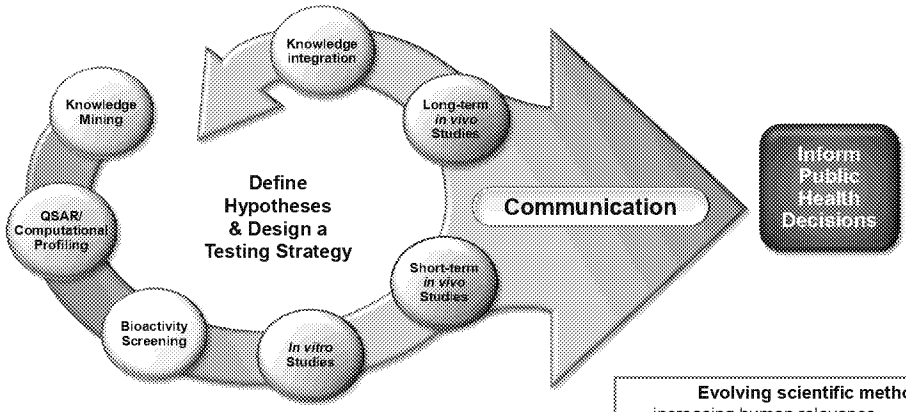
- Define and build a strategic assessment pipeline for key health effects
- Understand the mechanism / mode of action (MOA)
- Increase confidence in the predictivity of MOA assessments
- Align our capability development to critical areas of public health concern

Cardiovascular

Carcinogenesis

Developmental Neurotoxicity

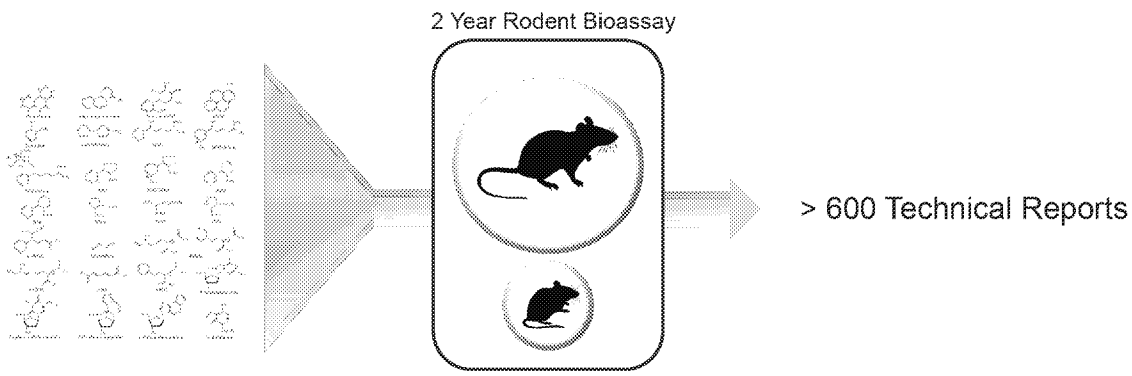
Strategic pipeline of capabilities



Evolving scientific method

- increasing human relevance
- hypothesis-driven assessments
- move from 'testing' to answering questions

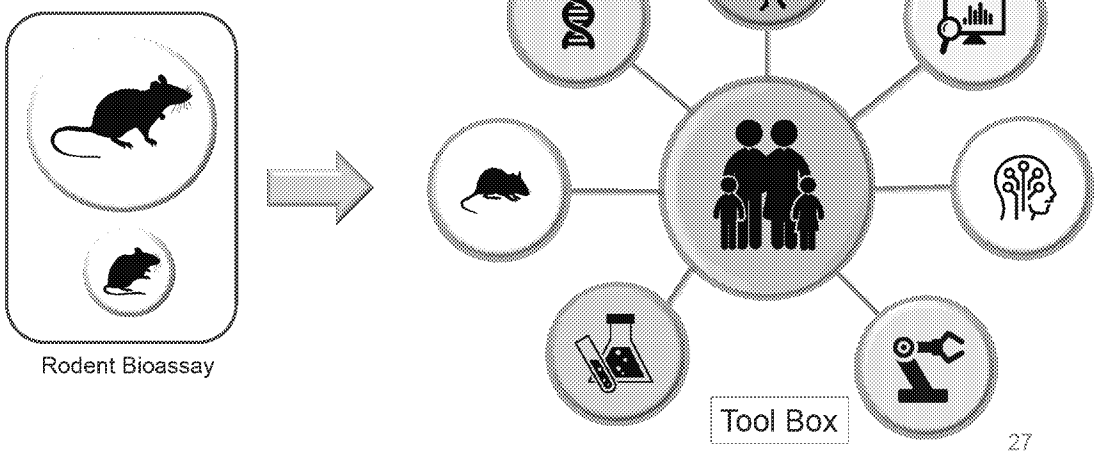
Carcinogenicity Testing Program @DNTP

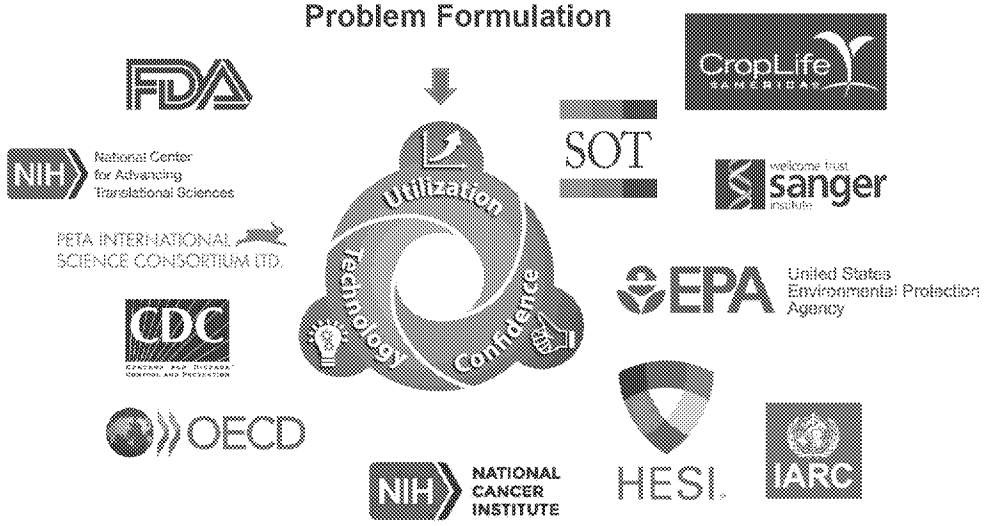


Challenges

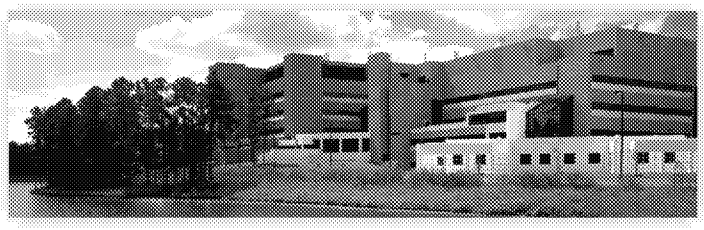
- Practicality:
  - >~8 years from Nomination to Report
  - 1$M
  - ~1000 animals per study
- Human Relevance:
  - Results are frequently positive but potentially irrelevant to human cancer risk for reasons such as dose, mode of action, and species specificity
  - Insufficient to inform low dose risk
  - Tissue concordance / coverage of human cancers
  - **Very little incorporation of human cancer biology in 50+ years**

Advancing Carcinogenicity Assessment

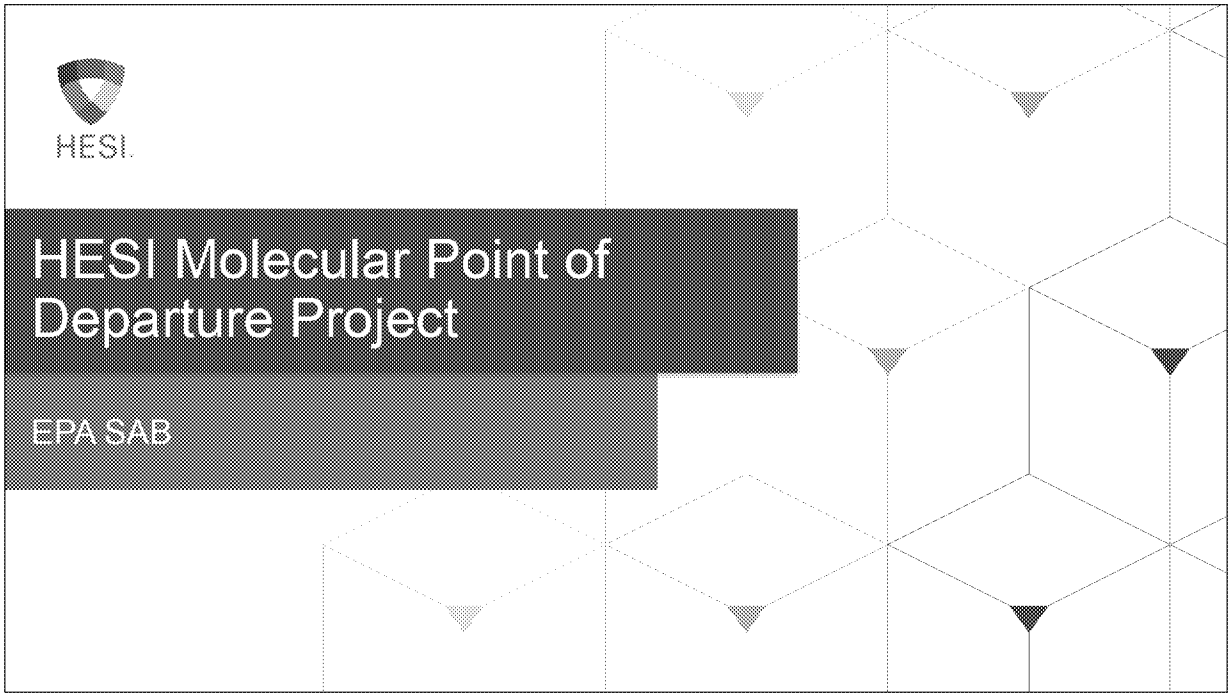



Problem Formulation
FDA
NIH National Center for Advancing Translational Sciences
PETA INTERNATIONAL SCIENCE CONSORTIUM LTD.
CDC
OECD
Utilization
Confidence
Technology
SOT
CropLife
sanger
EPA United States Environmental Protection Agency
HESI
IARC
NIH NATIONAL CANCER INSTITUTE

Thank You!



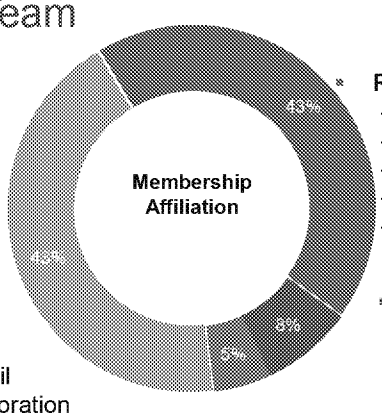
warren.casey@nih.gov


HESI
HESI Molecular Point of Departure Project
EPA SAB

HESI – eSTAR Committee

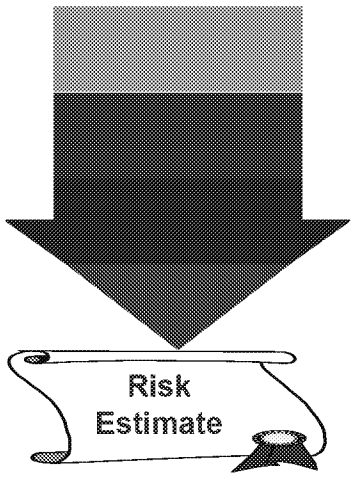
- The Health and Environmental Sciences Institute (HESI) engage global scientists from academia, government and industry to identify and resolve global health and environmental issues.
- eSTAR committee's mission is to develop and deliver innovative systems toxicology approaches for risk assessment.

eSTAR: Molecular POD Team



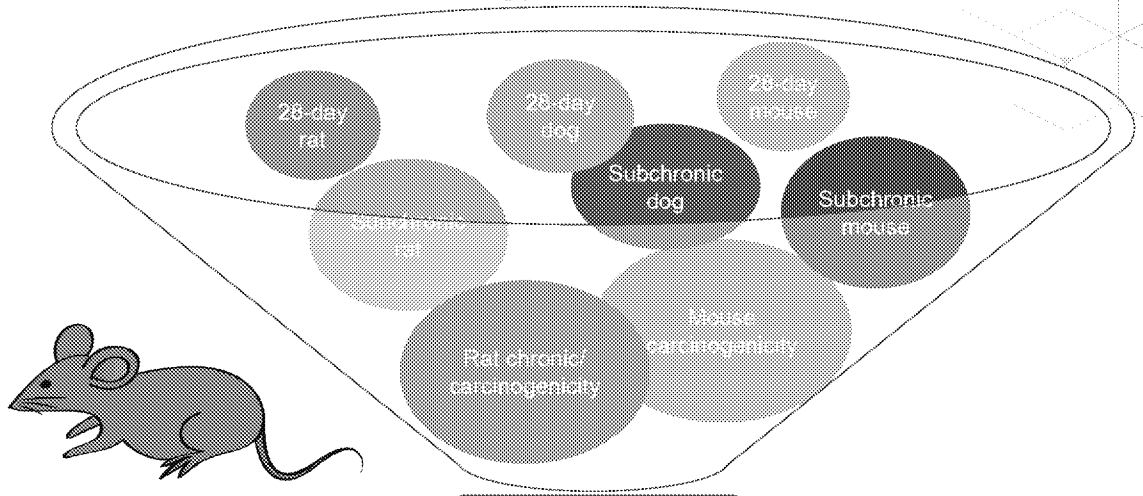
- **Regulatory/Government**
  - Health Canada
  - EPA
  - European Commission
  - NIEHS
  - NTP
- **Academic**
  - Indiana University
  - McGill University
  - University of North Carolina (UNC)
- **Industry**
  - Bayer
  - Corteva
  - ExxonMobil
  - FMC Corporation
  - GlaxoSmithKline
  - Janssen
  - Syngenta
- **Other/Consulting**
  - Juberg Toxicology Consulting
  - JLS Paradox Found Consulting

Safety Assessment Process



- Apical Effect and Hazard Identification
- Dose Response and Point of Departure (POD) derivation
- Exposure Assessment
- Risk Characterization

General Mammalian Toxicology Studies



Result Is a Point of Departure Value

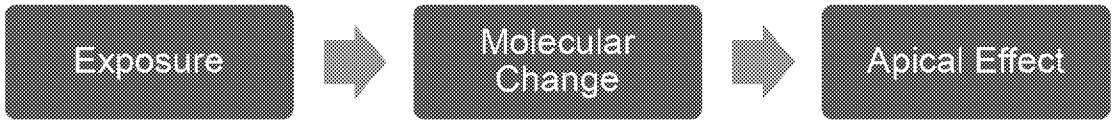
DART, mutagenicity, etc not illustrated

Challenges with Current Regulatory Testing Practices

- For environmental, industrial, and agricultural chemicals, a point of departure is needed to conduct human health risk assessments.
- Current regulatory testing practices are resource intensive (too many animals, too much time, too much money) which limits chemical testing throughput.
  - Traditionally, points of departure are derived from apical endpoints from subchronic and chronic toxicity studies.
- **How can we change the regulatory testing paradigm to reduce animals (3Rs), decrease time, increase throughput, while still protecting human health?**

All Apical Effects Result From A Prior Change At The Molecular Level

Generic Adverse Outcome Pathway



If a method *comprehensively* queries molecular change, it follows that this method can capture all possible apical effects.

HESI eSTAR POD Problem Statement

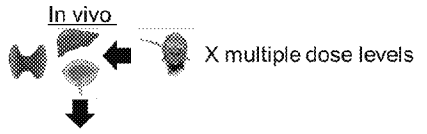
Develop a framework to derive an *in vivo* transcriptome POD for use in chemical risk assessment that will produce a human health-protective POD **without** needing to link the transcriptomic change with a specific adverse effect, mechanism, or mode of action.

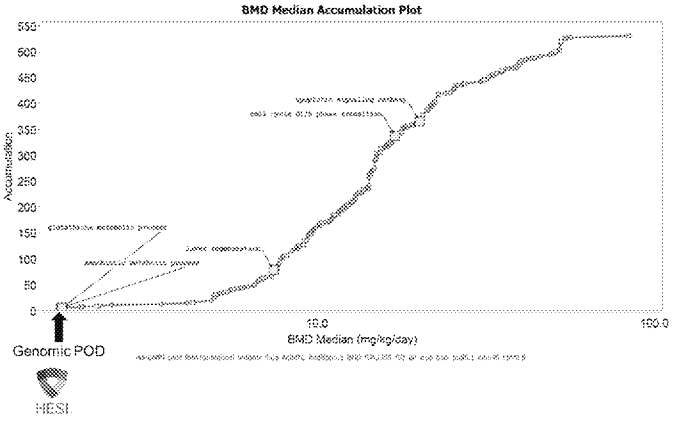
Goal is to identify a human health-protective chronic/cancer POD from a short-term study.

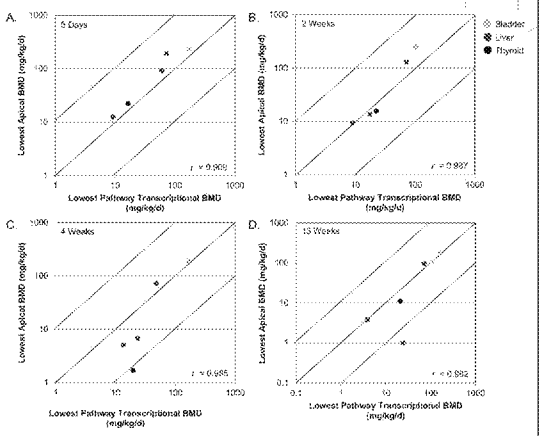
Existing evidence that short-term in vivo transcriptomic PODs closely approximate (3-10X) chronic/cancer study apical PODs.

Short-Term Study Genomic POD….Lets Test the Hypothesis

Study design (Thomas et. al., 2013)

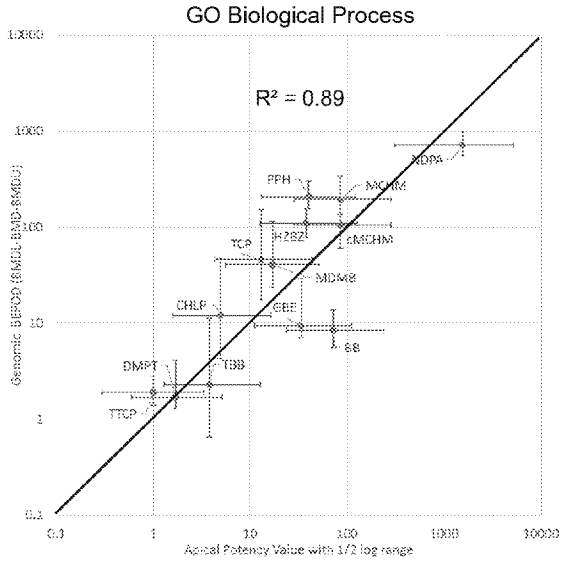






Thomas et. al., Tox Sci, 2013

Apical vs 5-Day Genomic POD



HESI

https://ntp.niehs.nih.gov/ntp/results/pubs/rr/reports/rr05_508.pdf

Case Studies

Temporal Concordance Between Apical and Transcriptional Points of Departure for Chemical Risk Assessment

Russell S. Thomas,*,† Scott C. Wesselkamper,‡ Nan Ching Wang,§ Q. Jay Zhao,∥ Dan D. Petersen,† Jason C. Lambert,† Jo Chen,§ Longtong Yang,† Eric Healy,† Michael B. Black,† Harvey J. Clewell III,† Bruce C. Allen,† and Melvin E. Andersen†

Cross-Species Transcriptomic Analysis of Mouse and Rat Lung Exposed to Chloroprene

Russell S. Thomas,*,† Matthew W. Himmelstein,† Harvey J. Clewell III,† Yuching Yang,† Eric Healy,† Michael B. Black,† and Melvin E. Andersen†

Genomic Signatures and Dose-Dependent Transitions in Nasal Epithelial Responses to Inhaled Formaldehyde in the Rat

Melvin E. Andersen,* Harvey J. Clewell, III, Rebecca Bermudez-Hernandez, Carole A. Willson, and Russell S. Thomas

Integrating pathway-based transcriptomic data into quantitative chemical risk assessment: A five chemical case study

Russell S. Thomas[a,*], Harvey J. Clewell III[a], Bruce C. Allen[a], Longtong Yang[a], Eric Healy[a], Melvin E. Andersen[a]

Case study on the utility of hepatic global gene expression profiling in the risk assessment of the carcinogen furan

Anna Francina Jackson[a,b], Andrew Williams[a], Leslie Recio[c], Michael D. Waters[c], Julie B. Fostel[b], Carole L. Yauk[a,b]

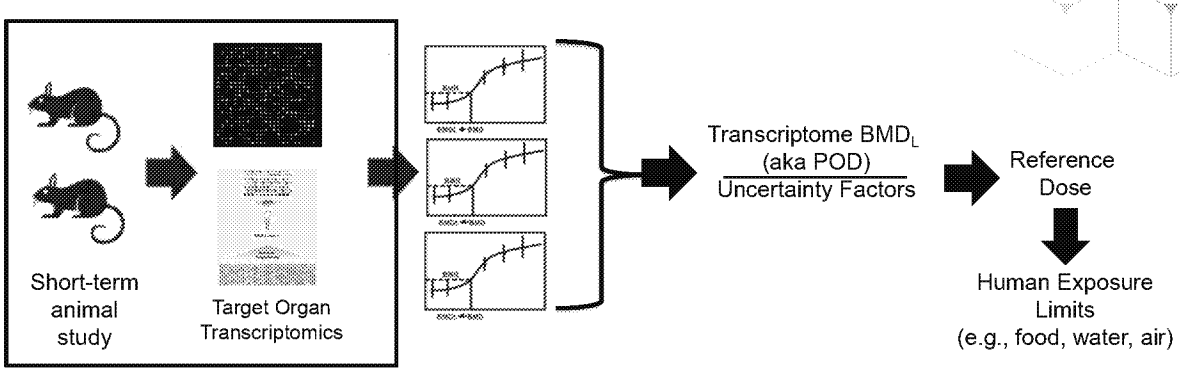
Integrating toxicogenomics into human health risk assessment: Lessons learned from the benzo[*a*]pyrene case study

Nikolai L. Chepelev, Ivy D. Moffat, Sarah Labib, Julie Bourdon-Lacombe, Byron Kuo, Julie K. Buick, France Lemieux, Amal I. Malik, Sabina Halappanavar, Andrew Williams & Carole L. Yauk

Comparison of toxicogenomics and traditional approaches to inform mode of action and points of departure in human health risk assessment of benzo[*a*]pyrene in drinking water

Ivy Moffat, Nikolai L. Chepelev, Sarah Labib, Julie Bourdon-Lacombe, Byron Kuo, Julie K. Buick, France Lemieux, Andrew Williams, Sabina Halappanavar, Amal I Malik, Mirjam Luijten, Jiri Aubrecht, Daniel R. Hyduke, Albert J. Fornace Jr, Carol D. Swartz, Leslie Recio & Carole L. Yauk

A New Paradigm for Risk Assessment Using Transcriptomics

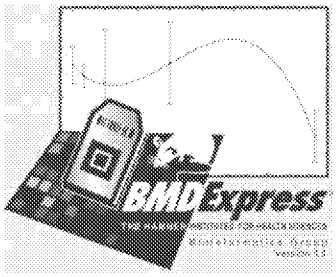


HESI

Adapted from Thomas et.al., Mut. Res., 2012

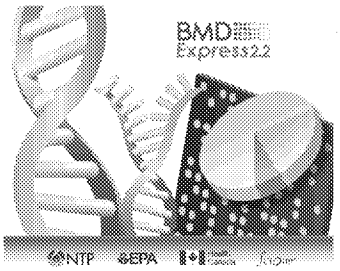
Software to Democratize the Analysis Approach

2007



2015
Hamner to NTP

2018



BMC Genomics

Software

BMDExpress: a software tool for the benchmark dose analyses of genomic data

Longlong Yang[1], Bruce C Allen[2] and Russell S Thomas[3,4]

Gene expression

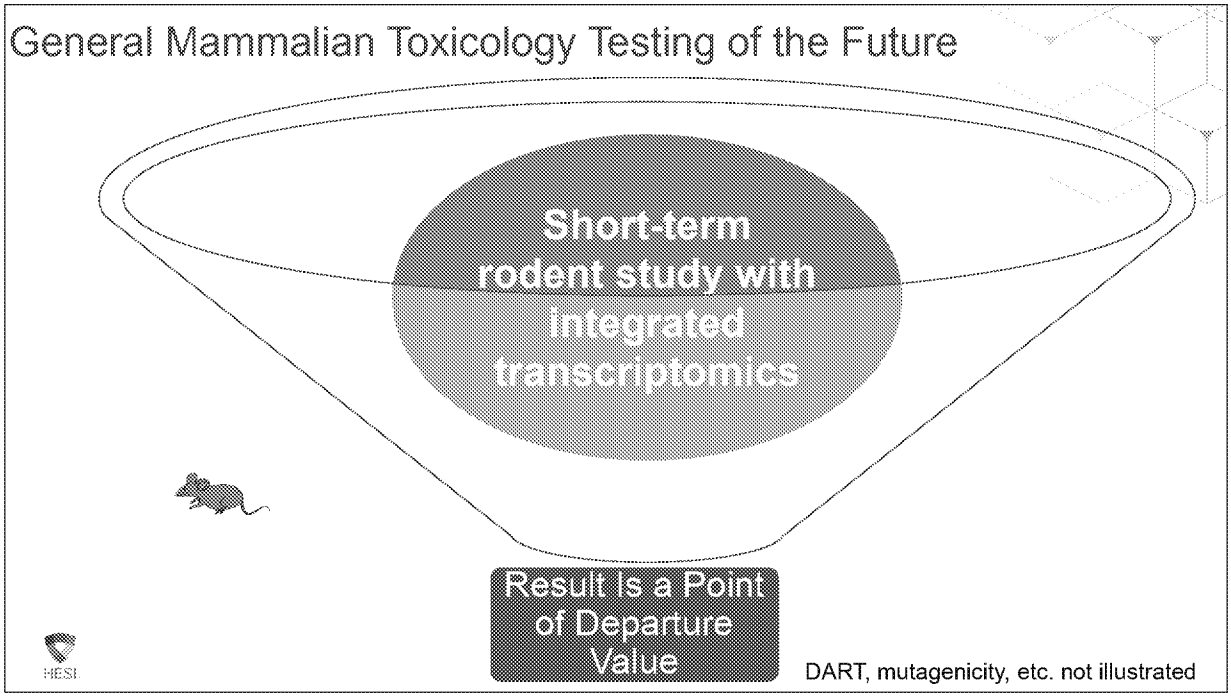
BMDExpress 2: enhanced transcriptomic dose-response analysis workflow

Jason R. Phillips[1], Daniel L. Svoboda[1], Arpit Tandon[1], Shyam Patel[1], Alex Sedykh[1], Deepak Mav[1], Byron Kuo[2], Carole L. Yauk[3], Longhong Yang[4], Russell S. Thomas[4], Jeff S. Gift[5], J. Allen Davis[6], Louis Olszyk[7], B. Alex Merrick[8], Richard S. Paules[9], Fred Parham[9], Trey Saddler[9], Ruchir R. Shah[1] and Scott S. Auerbach[5,*]

HESI

What Still Needs To Be Addressed?

- Develop a consensus on analysis methods and POD determination
  - What represents a biological response appropriate for risk assessment?
  - Analysis methodology
    - Better separate signal from noise
    - Overall reproducibility of findings
  - Overall study design
    - Dose groups/size/selection, technology, organs examined, exposure duration
- Greater accuracy in approximation of guideline study PODs
  - 3-fold vs 10-fold


General Mammalian Toxicology Testing of the Future
Short-term rodent study with integrated transcriptomics
Result Is a Point of Departure Value
HESI
DART, mutagenicity, etc. not illustrated

Evaluation of Pesticides with Established Liver Tumor Modes of Action

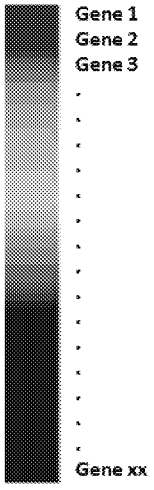
Chris Corton

Center for Computational Toxicology and Exposure
US-Environmental Protection Agency
Research Triangle Park, NC

Disclaimer

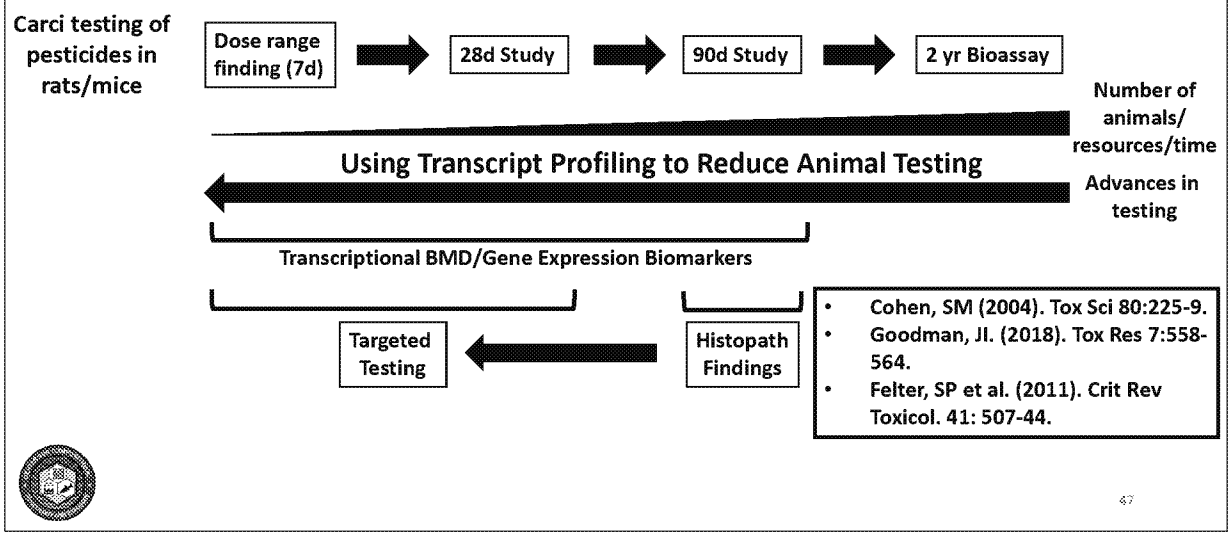
- The views expressed are those of Dr. Chris Corton and do not reflect US-EPA policy or product endorsement by the US-EPA.

Gene Expression Biomarkers



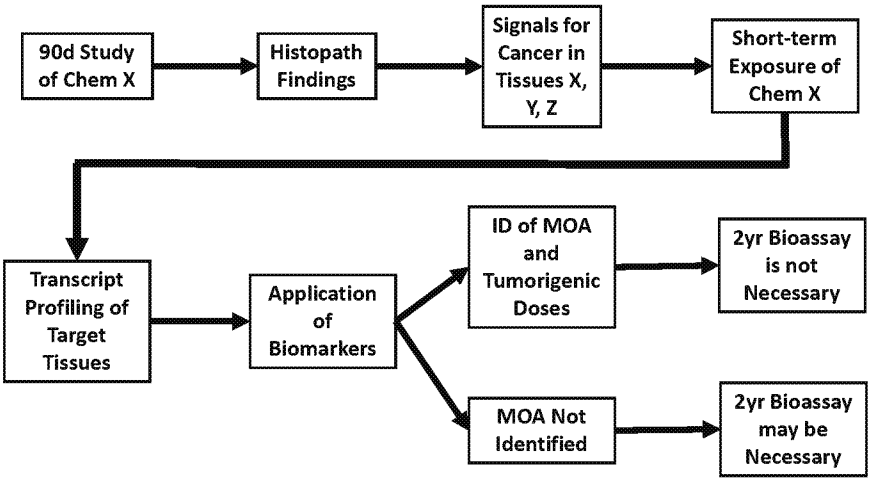
- List of genes and associated fold-change values or ranks
- Measures a molecular initiating event or key event in an adverse outcome pathway using transcript profiling
- Can be used to identify the mechanism of toxicity of a chemical
- Biomarkers that predict MIEs in mouse liver: AhR, CAR, PPARα, Nrf2, Stat5b, SREBP (multiple publications)
- Biomarkers that predict MIEs in rat liver: DNA damage, AhR, CAR, ER, PPARα, Cytotoxicity (Corton et al. (2020). Tox Sci. In press.)
- Levels of biomarker activation are associated with liver tumor incidence (Hill et al. (2020). Tox Sci. In press.

Applications of Genomic Tools to Chemical Testing

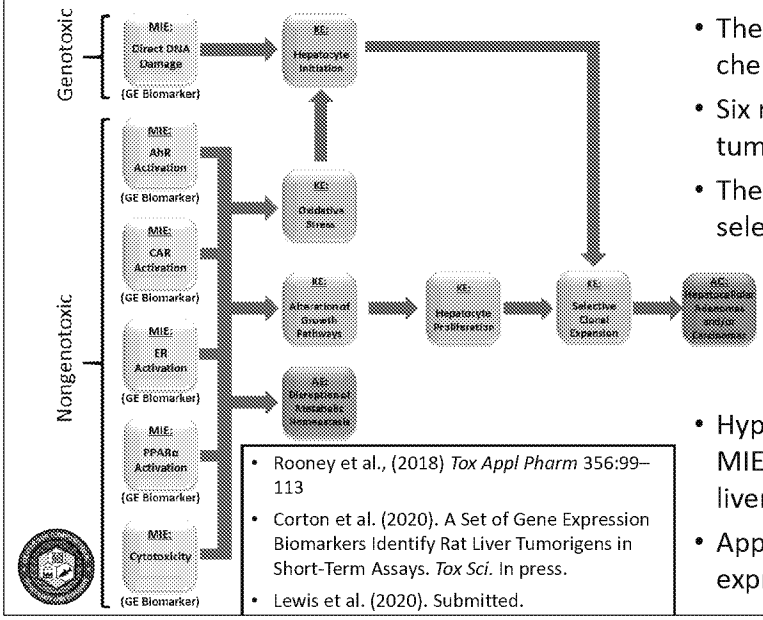


- Cohen, SM (2004). Tox Sci 80:225-9.
- Goodman, JI. (2018). Tox Res 7:558-564.
- Felter, SP et al. (2011). Crit Rev Toxicol. 41: 507-44.

Applications of Genomic Tools to Chemical Testing



Major Adverse Outcome Pathways That Lead to Rodent Liver Tumors



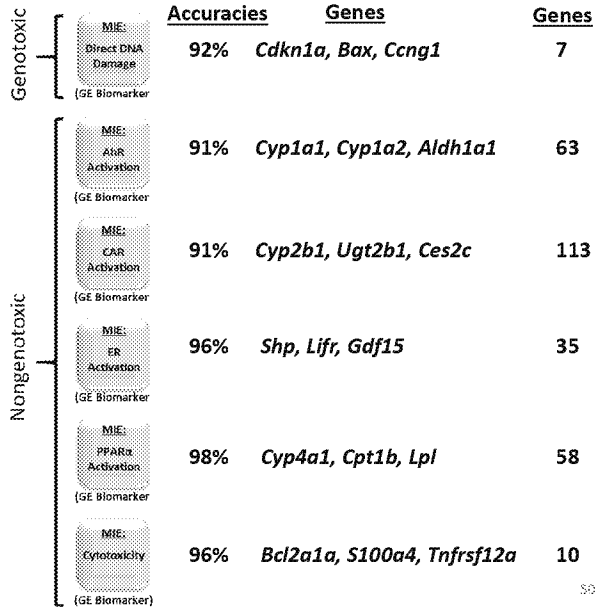
- Rooney et al., (2018) *Tox Appl Pharm* 356:99–113
- Corton et al. (2020). A Set of Gene Expression Biomarkers Identify Rat Liver Tumorigens in Short-Term Assays. *Tox Sci.* In press.
- Lewis et al. (2020). Submitted.

- The liver is the most frequent target of chemical tumorigens
- Six major AOPs lead to rodent liver tumors
- The AOPs converge on the key event of selective clonal expansion

- Hypothesis: measurement of the six MIEs will be sufficient to predict rodent liver tumors
- Approach: measure MIEs with gene expression biomarkers

Predictive Accuracies of Six Gene Expression Biomarkers

- All biomarkers have balanced accuracies above 90%
- Genes identified are known to be regulated by the MIE



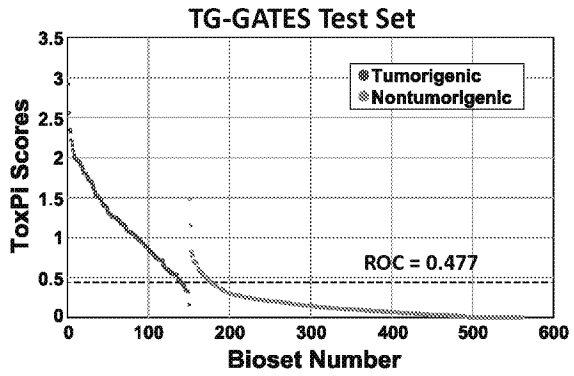
	Balanced Accuracies	Examples of Biomarker Genes	Number of Genes
MIE: Direct DNA Damage (GE Biomarker)	92%	*Cdkn1a, Bax, Ccng1*	7
MIE: AhR Activation (GE Biomarker)	91%	*Cyp1a1, Cyp1a2, Aldh1a1*	63
MIE: CAR Activation (GE Biomarker)	91%	*Cyp2b1, Ugt2b1, Ces2c*	113
MIE: ER Activation (GE Biomarker)	96%	*Shp, Lifr, Gdf15*	35
MIE: PPARα Activation (GE Biomarker)	98%	*Cyp4a1, Cpt1b, Lpl*	58
MIE: Cytotoxicity (GE Biomarker)	96%	*Bcl2a1a, S100a4, Tnfrsf12a*	10

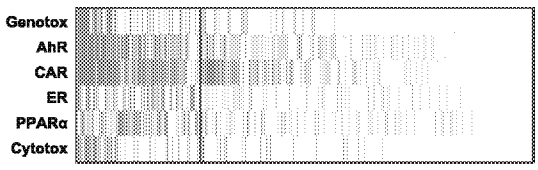
- Rooney et al., (2018) *Tox Appl Pharm* 356:99–113
- Corton et al. (2020). A Set of Gene Expression Biomarkers Identify Rat Liver Tumorigens in Short-Term Assays. *Tox Sci.* In press.

Predictions of Six MIEs Identifies Liver Tumorigens

- Used a combination of ToxPi and Receiver Operating Curves to examine a test set of chemicals
- 90% sensitivity, 97% specificity, and a **balanced accuracy of 93%**

- Out of 38 rat liver tumorigens, only two (5%) were not predicted (acetamide, ethionine)
  - These chemicals may work through different AOPs
  - Allows a better understanding of the weaknesses of the approach

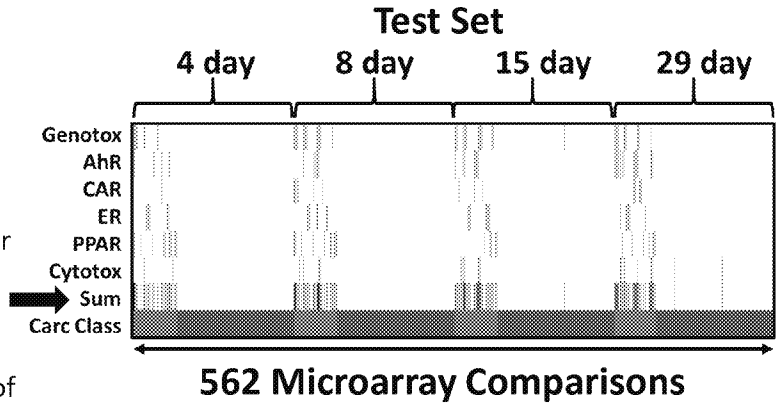




- From Corton et al. (2020). A Set of Gene Expression Biomarkers Identify Rat Liver Tumorigens in Short-Term Assays. *Tox Sci.* In press.

Biomarker Activation Levels Accurately Predict Liver Tumors

- Identified activation levels associated with tumor induction from a training set and then applied to a test set
- Each red line is a chem-dose condition in which the biomarker tumorigenic level is surpassed
- Most of the tumorigenic conditions exceed one or more of the 6 activation levels
- Activation levels rarely exceeded in any of the nontumorigenic conditions



- **Test set: 100% sensitivity, 94% specificity, and a balanced accuracy of 97%**

Tumorigenic
Nontumorigenic

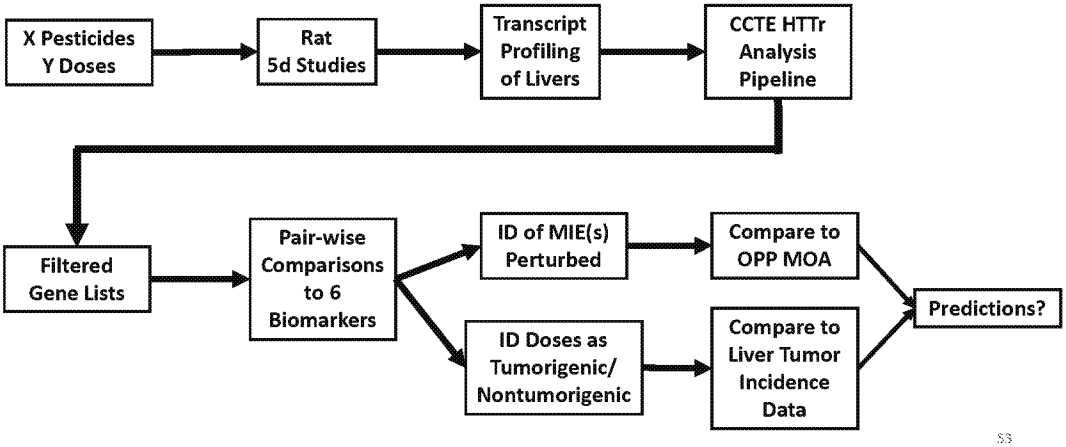
52

From Hill et al. Tox Sci In press

OPP-CCTE Project

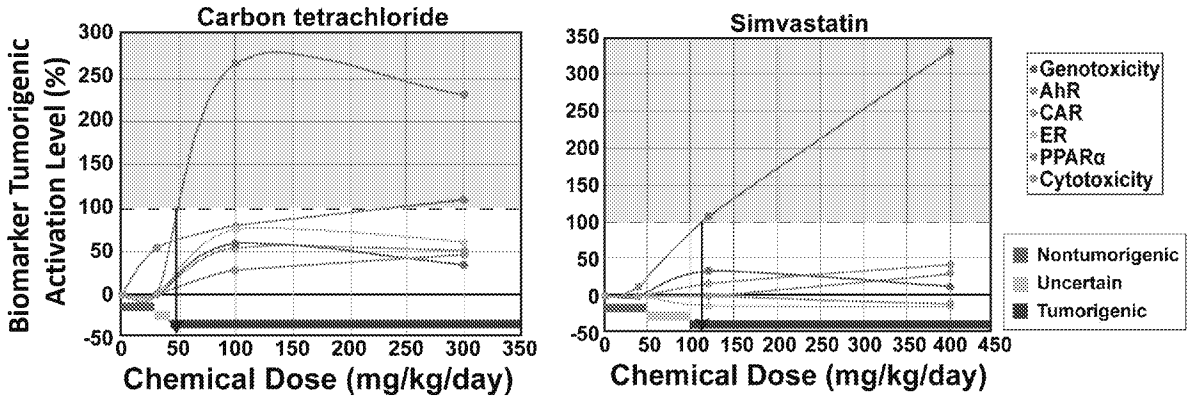
Using biomarkers can we predict from short-term studies of pesticides:

- Mode of action by which the tumors would arise?
- Chemical-dose combinations that will cause tumors?



Application of Biomarkers and Activation Levels to Model Liver Tumorigens

- Chemicals examined in the TG-GATES study in male rats for 15d at 3 doses



- Approach identifies the MOA and the lowest tumorigenic dose

From Hill et al. Tox Sci. In press

Summary

- An AOP-guided computational approach can be used to identify liver tumorigens in prospective studies
  - Two sets of tools to apply to toxicogenomic studies
    - Gene expression biomarkers
    - Activation levels associated with tumor induction

Emerging Systems Toxicology for the Assessment of Risk Committee (eSTAR)
Carcinogenomics Subcommittee
Co-chairs: Chris Corton, EPA
Keith Tanis, Merck

- The 6 biomarkers could identify chemical-dose pairs from tumorigenic treatments (balanced accuracy = 93%).
- Biomarker activation levels could identify chemical-dose pairs from tumorigenic treatments (balanced accuracy = 97%).
- Will perform a case study on pesticides with known MOA to evaluate the application of the approach.

SS

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Abigail Jackson
Katharine Korunes

National Center for Advancing Translational Sciences

Ruili Huang
Menghang Xia

Indiana Biosciences Research Institute

Jeff Sutherland
Jim Stevens

Kinetically-Derived Maximum Doses

Cecilia Tan, MS, MBA, Ph.D.
Senior Research Scientist
US Environmental Protection Agency, Office of Pesticide Programs
Tan.cecilia@epa.gov / +1-919-541-2542 / +1-919-610-2174
Consultation with the Science Advisory Board
June 24, 2020

Outline

- Incorporating Kinetic Data/Model in Risk Assessment
- Kinetically-Derived Maximum Dose (KMD)
  - Definition
  - Implication in Risk Assessment
- Case Study
- KMD-related Efforts

Kinetics in Risk Assessment: Dose Makes the Poison

- Risk assessment is the characterization of the potential adverse effects of human **exposures** to environmental **hazards** (NRC, 1983)
- Kinetics determines the movement of a chemical into, through, and out of the body; the time course of a chemical's absorption, distribution, metabolism, and excretion
- The internal target tissue dose determines the initiation and degree of toxicological responses
- Kinetics connects exposures to hazards

Value of Kinetic Data/Models

- Support smarter testing strategies
  - Reduce & Replace: eliminate duplicative testing or unnecessary studies
  - Refine: lessen animal suffering by not testing at doses that cause overt toxicity
- Quantify and reduce uncertainty in risk assessment
- Evaluate consistency with mode of action hypothesis
- Extrapolate points of departure across species, routes, life-stages, etc.

Examples of risk assessment applications in OPP

- Using physiologically based pharmacokinetic (PBPK) models to replace the use of default uncertainty factors for inter-species extrapolation, route-to-route extrapolation, and age-specific extrapolation
- Using PBPK models to estimate scenario-specific points of departure
- Using *in vitro* and *in vivo* dermal absorption measurement to adjust route-specific points of departure
- Using *in vitro* metabolism data to understand dose-response difference across species or life-stages
- Using kinetic data to interpret dose-response data or select doses in animal toxicity studies – kinetically-derived maximum dose (KMD) approach

KMD Definition

- KMD is the highest dose at, or slightly above, the point of departure from linear kinetics
- Non-linear kinetics can arise from various factors, such as saturation of absorption, metabolism, protein binding, excretion, resulting in chemical concentrations in the body to be disproportionally high or low relative to the change in external dose

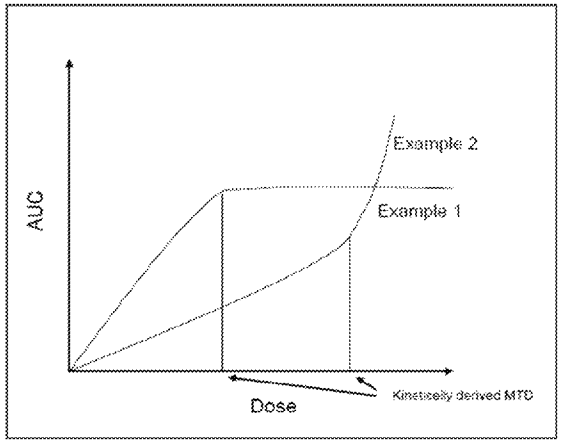

Figure adapted from 2008 REACH Guidance, Figure R.7.12-2

KMD Implications

- When internal dose becomes disproportionally low relative to the change in external dose, "there is little point in increasing administered dosage if it does not result in increased plasma or tissue concentration" (ICH S5)

- When internal dose becomes disproportionally high relative to the change in external dose, "exposures in rodents, greatly in excess of the intended human exposure, might not be relevant to human risk; because they so greatly alter the physiology of the test species" (ICH S1A, S1B, S1C)

Case Study – Weight of Evidence Approach

- Study purpose: Understand if lung tumors observed in male mice at high dose (60 ppm) of telone are due to saturation of metabolic clearance
- Multiple lines of evidence suggest that systemic exposures in mice become non-linear at 30 ppm or above
  - Both a hockey-stick model and a power model conclude that area under the curve (AUC) of blood concentrations become non-proportional to external dose between 30-40 ppm
  - The cis- and trans-isomers of telone changes from 0.13 to 0.2 between the external concentrations of 40-60 ppm
  - The glutathione(GSH)-dependent metabolism of telone results in significant depletion of GSH at external dose 30 ppm and above

An International Effort – Developing Best Practices

- Under the MOU between EPA and Health and Environmental Sciences Institute (HESI), a KMD project is initiated in 2020 by the HESI PBPK Committee
  - Develop best practices and guidance on the KMD analysis
  - Discuss if and how KMD can be applied in the context of risk assessment
  - Identify situations where the use of KMD might be limited or prohibited
- A 3-day virtual workshop, co-sponsored by NICETAM, USEPA, and HESI, will be held on October 6-8, 2020
  - Address commonly raised technical and scientific issues related to KMD
  - Discuss best practices and lessons learned
  - Discuss the possible applications and limitations of KMD

Summary

- Reduce: Develop weight of evidence (WOE) approach for waiving chronic/carcinogenicity studies
  - Please comment on the clarity and completeness of the proposed risk-based WOE approach
  - Please comment on the draft case study provided
- Replace: Develop NAMs for chronic/carcinogenicity studies
  - Please comment on the direction and scope of the three collaborative projects
- Refine: Use kinetically-derived maximum dose (KMD) approach to refine and interpret chronic/carcinogenicity studies
  - Please comment on the current KMD-related activities and suggest additional activities, if any